Using Digital MICROSC

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Studying live cultures using a digital dissection microscope intertwines genetics, anatomy, and physiology

Studying *Caenorhabditis elegans* (*C. elegans)* live cultures provides excellent opportunities for authentic inquiry in a high school anatomy and physiology or other biology lab course. The anatomy of *C. elegans,* a free-living roundworm found in soil throughout the world, can be observed quite easily because of its transparent nature. The organism is a favorite among researchers because it shares many of the essential biological characteristics of vertebrates, yet it can be handled as a microorganism with a life span of two to three weeks. Using a digital dissection microscope, a student can photograph the organism during various stages of development and study and analyze the images.

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OPY

A successful activity in my high school anatomy and physiology laboratory course uses the digital dissection microscope to study live cultures of *C. elegans*. We adapted several laboratory protocols that address the National Science Education Standards (NRC 1996); encourage the use of inquiry; and intertwine genetics, modern molecular biology, anatomy, and physiology in one unit. Students design their own experiments and participate in inquiry-based lessons. Incorporating digital microscopes in the anatomy and physiology laboratory has increased students' ability to compare and contrast stages of development and study the behavior of *C. elegans* in greater depth. *C. elegans* is ideal to use in high school laboratories because of the organism's short life cycles, small size, and inexpensive maintenance materials.

Using a digital video microscope.

Digital microscopes vary in price and capabilities and many grants can defer the cost of obtaining several microscopes. Although prices have come down, the cost of digital dissecting microscopes can be prohibitive. It is possible to conduct these experiments with a combination of standard stereo light microscopes and digital video microscopes if students are assigned to complete different tasks and rotate using the available digital microscope(s). If digital video microscopes are not available, standard stereomicroscopes can be used.

These experiments can also be conducted using a single video microscope. If a projection system is available, the teacher can use the video microscope as a demonstration tool, or hard copies of images can be printed and shared with students. This will aid students who are working on nondigital microscopes and will not have the ability to analyze their videos and pictures at a later time.

Light micrograph of *Caenorhabditis elegans* (left).

Background on *C. elegans*

Genetic details

The relatively small genome (97 megabases) of the nematode *C. elegans* has been sequenced and its entire nervous system has been mapped and consists of 302 neurons (Jorgensen and Mango 2002). Scientists have determined that about 35% of *C. elegans* genes have human homologs (Kamath et al. 2000). Through the process of RNA interference (RNAi)—a sequence-specific mechanism of messenger RNA degradation by double-stranded RNA—scientists were able to study the similarities of homologous genes found in both humans and *C. elegans* and therefore determine the function of such genes (Matzke, Matzke, and Kooter 2001).

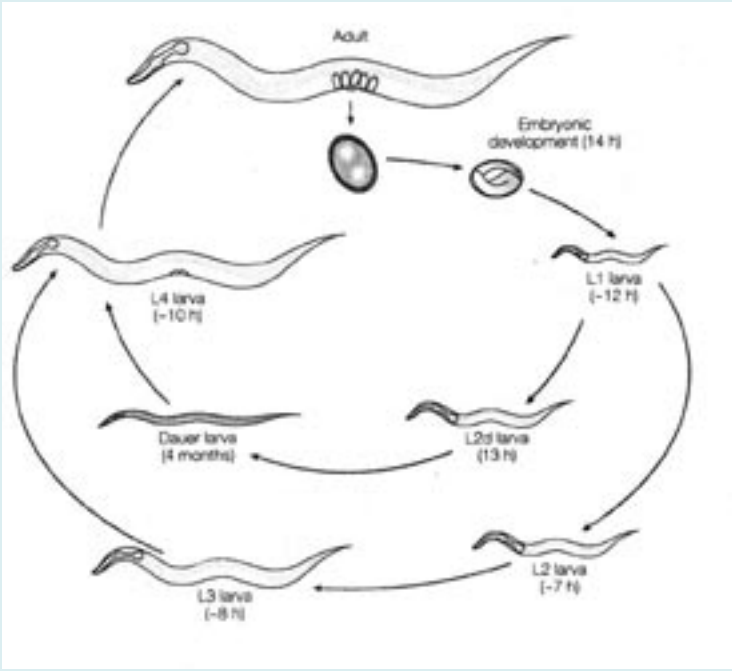
Anatomy and development

C. elegans is about 1 mm long as an adult and has five pairs of autosomes (chromosomes I, II, III, IV, V) and the sex chromosome X. There are two sexual forms: a self-fertilizing hermaphrodite and a male. Hermaphrodites, the predominant sexual form, contain two sex chromosomes (XX) (Kim et al. 2001). Each animal produces about 300 progeny. Self-fertilization leads to homozygosity of alleles; therefore, individual worms are considered genetically identical (as long as mutations have not occurred) (Hansen and Pilgrim 1999).

FIGURE 1

Life cycle of *C. elegans*.

Reprinted with permission from Jorgensen and Mango 2002.



A developmental switch occurs during *C. elegans* development. Sperm are produced and stored in the spermatheca and then oocytes are produced and mature in a syncytium. After fertilization, the eggshell is added. Fertilization takes place as maturing oocytes are squeezed through the spermatheca. Eggs are then laid through the vulva at about the 40-cell stage (Hansen and Pilgrim 1999).

Life cycle

C. elegans has a temperature-dependent life cycle. It can go through the reproductive life cycle in 5.5 days at 15°C, 3.5 days at 20°C, and 2.5 days at 25°C. Figure 1 illustrates *C. elegans* in four larval stages—LI, L2, L3, L4 (Ewbank 2002).

If conditions are not favorable, the worms will enter a *dauer* stage, which occurs between the L1 and L2 stage of development. The dauer stage is a developmentally arrested dispersal stage that may be formed under conditions of starvation or overcrowding, in which the larvae mouths become plugged and they cannot eat. In this stage, worms can remain viable for up to three months. Adult worms have an average life span of two to three weeks. When food and adequate space are available, the worms will enter the L4 stage and live for about 15 days (Ewbank 2002).

Mutant strains

It is easy to find motional defective worms to use in experiments. For example, the phenotype of worms with a selected missense mutation in the *unc-54* gene that encodes a muscle myosin, a major component of thick muscle filaments, has a limp paralyzed phenotype, yet the worm's muscle structure is not destroyed. The *unc-7* mutant also lends itself to the study of locomotion because these mutants are unable to propagate the smooth sinusoidal body bends seen in the wild-type worms (Starich, Hermann, and Shaw 1993). Instead, *unc-7* worms display sharp irregular movement easily observed in the laboratory.

By conducting these experiments and observing the worms, students can determine the relationship between muscle structure and muscle function. For example, by comparing wild-type to mutants, students realize that possessing the same muscle structure does not mean the muscles will perform the same function equally. Furthermore, students can apply this understanding to some human diseases, such as certain human heart diseases and muscular dystrophy.

Laboratory investigations

During this project, students worked in teams of four and related their research to information found in key articles discussed in class (see the "References" list at the

Acquiring and caring for strains of *C. elegans*.

Different strains of *C. elegans* can be obtained from the Caenorhabditis Genetics Center at the University of Minnesota *(http://biosci.umn.edu/CGC/Strains/request.htm)* at no charge to educational institutions. The request must be made in writing and should indicate that the request is for educational purposes. The university will also pay the shipping charges for the worms when they are sent to educational institutions.

In nature, *C. elegans* feeds on bacteria and fungi; therefore, in lab experiments this organism can be maintained in nematode growth medium (NGM) plates and fed *Escherichia coli* (Kamath et al. 2000). *C. elegans* cultures can be maintained using plates containing Nematode growth media and the *E. coli* strain OP50. In order to maintain the culture, worms must be transferred from existing plates. *C. elegans* can be visualized using the dissection microscope and transferred by removing a section of the existing plate with a sterile scalpel. Worms should be transferred every two to three days in order to see the different stages of development.

For more information, visit *www.wormbook.org/chapters/preprints/WormMethods/Forwardgenetics/StrainMaintain.pdf.*

Observing *C. elegans* using a digital microscope.

Materials and equipment

- One worm plate of each type (wild type and unc)
- Digital microscope
- Computer with adequate space for image storage. (The size of the capture file is important when conducting this experiment). If possible teachers should contact their network administrator to determine the best location for image storage (hard drive, network server, CD-R, DVD).

Day 1: Digital microscope and worm observation

1. Observe your worm plate using the digital microscope.
2. Capture still images and video of the following:
   a. Basic anatomical structure
   b. Movement of *C. elegans* worms
   c. How *C. elegans* obtains food

Note: Remember to check disk space before saving pictures or video. If you run out of disk space you will need an additional disk.

When you have completed steps 1 and 2:

3. Store your worm-containing plates upside-down on the front lab bench at room temperature.

Note: Since *C. elegans*' reproductive life cycle is very brief at room temperature (2 to 3 days), after about one week your plates will be very crowded with two to three generations of worms. (You will need to obtain additional plates if you are planning additional experiments.)

Lab questions (to be completed on a separate sheet of paper)

1. What is the purpose of the investigation you completed today?
2. What organism are we working with? Describe its anatomical characteristics and general life cycle.
3. Why is *C. elegans* a good organism to work with in a classroom and in research?
4. What do *C. elegans* worms eat? How?
5. Describe the movement of *C. elegans*. Did you observe more than one type of movement? Explain. Why are there differences in the movement observed?
6. How fast do the worms move? Did they all have a uniform speed?
7. What would happen to the worms on the plate if we kept using them for two weeks? (Discuss the life cycle.)

Day 2: Observations and development

Obtain your worm plates and complete the following:

1. Obtain a new count (number of worms on plate).
2. Describe and capture still images of stages of development.
   Larvae
   Adult
   Adult with eggs
   Adult laying eggs (video)
3. Determine if there are both hermaphrodites and males in your worm plate.

When you have completed steps 1–3:

4. Store your worm-containing plates upside-down on the front lab bench at room temperature.

Lab questions

1. Were there more worms on your plate? If so, why?
2. How were you able to differentiate between the different stages of development? Are all of your worms the same age?
3. Did you have males and hermaphrodites in your worm plates?
4. Develop a hypothesis and experiment to test the movement of *C. elegans* in relation to environmental stimulus. Remember to include a control and only one variable to test. Create your hypothesis in an "If....then" format and include materials and methods.

Day 3: Independent environmental stimulus experiment

1. Obtain your approved hypothesis statement and experiment from the teacher. Read the comments for suggestions or changes.
2. Set up your experiment and obtain digital images.
3. Create a chart of results.

Write-up: What to include on the report

1. Hypothesis statement
2. Materials list
3. Methods (procedure and directions)
4. Results (charts, digital images, and written description)
5. Discussion (discuss how your results supported or did not support your hypothesis; discuss possible areas of error or limitations you encountered).

Extension: *C. elegans* experiment using mutant strains *(unc-7)*

Students will repeat the above experiments using the mutant strains to compare the behavior (locomotion, responses) of the wild-type to the uncoordinated mutant strains.

end of this article for reading assignments). The use of digital microscopes instead of the traditional microscope increased students' ability to review and spend more time analyzing the behavior of the organism (see "Using a digital video microscope," p. 51).

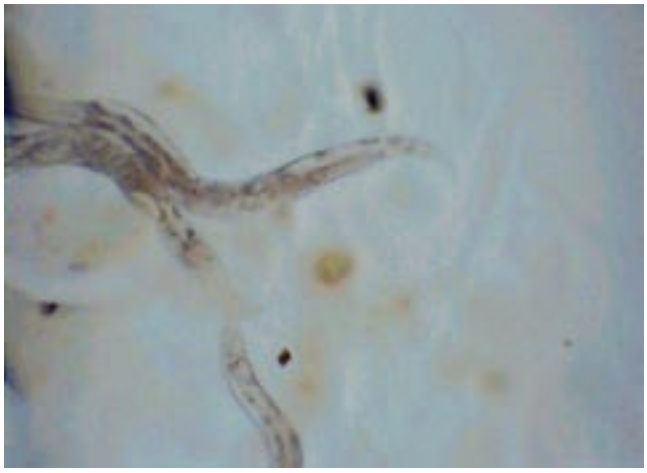
The overall project consisted of several laboratory exercises designed for students to learn to use the microscope, care and maintain the worms, and observe a behavioral characteristic selected by the teacher. Later in the project, students created a new experiment involving the worms, which allowed students to choose a characteristic in which they were interested, develop their own hypothesis, and design experiments. Students developed, set up, and conducted their own experiments in which the teacher became a facilitator, helping students as necessary. The variable tested in each experiment varied; however, students were limited to observing and examining behavioral characteristics. Student experiments ranged

FIGURE 2

A student's digital photo of *C. elegans*.

FIGURE 3

A student's digital photo of *C. elegans*.



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from observing the worm's behavior in relation to surface texture, light intensity, or temperature to determining the effect of different nutrients and vibration.

When their own digital images were used as assessments of their work, students took greater care thoroughly examining the specimen. Using the software and digital images, students identified structures and described functions associated with those structures. This created a direct connection between the topics discussed in class and students' laboratory experiments.

Using the digital microscopes greatly enhanced the developmental studies. On many occasions students would leave the classroom and many changes occurred in the organism in their absence. The digital microscopes allowed students to take pictures at scheduled intervals. Students programmed the microscope to take a series of pictures over one to two hours. Other students could not use the programmed microscope during that time. (It was not necessary to use the microscopes during every class period; therefore, this never affected the use of the microscopes by other anatomy and physiology classes.)

Figures 2 and 3 show two students' digital images of the worms. The pictures were stored on the computer hard drives and later analyzed. Students displayed greater interest in developmental changes when analyzing pictures of their cultures. Behavioral analysis using the digital microscopes also allowed students to capture images and video of wild-type and *unc-7* (locomotion mutants) worms. The use of the video feature on the digital microscope allowed students to capture a moment in time. This could not be done with a standard (nondigital) microscope.

Students acquired significant laboratory skills when conducting these activities. Students learned how to use microscopes, to distinguish the sexes, to characterize wild-type and mutant worms, to observe behaviors in different environments such as those in which there are different chemical substances, and to maintain cultures of *C. elegans*. Keeping a lab notebook may be helpful if students are to maintain their own worms. Students could also create posters of their projects and present them to their peers.

Student interest and excitement in my classroom increased during these experiments. These lessons provide a greater understanding and appreciation for science and scientific research through the integration of technology and analysis of *C. elegans* live cultures. The level of excitement in the classroom was enhanced by the use of a model organism. ■

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